ANTIOXIDANT ACTIVITY OF CRUDE EXTRACT FROM MANGOSTEEN *(Garcinia mangostana* Linn.) PERICARP ON THE LUNG RAT WHICH EXPOSURE BY CIGARETTE

THESIS

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ABSTRACT

Nabil Ahmed. 116100100111012. Food Technology Postgraduate, Faculty of Agricultural Technology, University of Brawijaya, Malang. Antioxidant activity of Crude Extract from Mangosteen (*Garcinia mangostana* Linn.) Pericarp on The Lung Rat which Exposure by Cigarette.
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Crude extract of mangosteen (Garcinia mangostana L) pericarp (CEMP) possess many biological and pharmacological activities. The aim of this study is to analyse the effect of CEMP consumption for 3 weeks on lipid peroxidation as shown by malondialdehyde (MDA) level in cigarette smoke exposed rats. Twenty five Wistar male rats were randomly divided into five groups, i.e.: a normal control group, an exposed cigarette control group and three treatment groups. The treatment groups either received 200; 400; and 600 mg CEMP/kg weighing of rats, respectively. The diets in the form of pellets were freely consumed (*ad libitum*) and were given for three weeks. Rats were exposed to cigarette smoke one time per day. Blood samples were taken on the last day for MDA analyses. Comparison of MDA levels was done by ANOVA's test on normal data. After 3 weeks treatment, the mean MDA levels between groups were significantly differences (P=0.000). On day 21th, the MDA levels on normal control, exposure by cigarette control and groups that supplemented with 0; 200; 400 and 600 mg CEMP / kg weighing of rats were 0.126 ± 0.02; 0.637 ± 0.04; 0.423 ± 0.03; 0.235 ± 0.03 and 0.136 ± 0.03 µg/mL, respectively. It is also interesting to point out the decrease of the lung morphological damage by CEMP treatment. These results indicate the beneficial effect of CEMP in the MDA level reducing on the rats that exposed by cigarette.

Keywords: crude extract of mangosteen pericarp (CEMP), cigarette, malondialdehyde (MDA), lipid peroxidation, rat

CONTENTS

TABLE LIST

FIGURE LIST

I. INTRODUCTION

1.1 Background

Smoking caused many dangerous diseases like lung cancer, tumors and others. Cigarette smoke contains more than 4000 elements and at least 200 of them are harmful to health. The main toxins in cigarettes are tar, nicotine, and carbon monoxide. In addition, cigarette smoke also contains other chemicals that are not less toxic such as ammonia, formic acid, formaldehyde, hydrogen cyanide, etc. (Ruslan, 1996). Until now, the number of smokers in Indonesia reached 27.6% of the total population of Indonesia. This has put Indonesia as the country's third biggest smokers in the world with total production amounting to 225 billion sticks of cigarettes per year (Nusantaraku, 2009).

The combustion of cigarettes can lead to the production of reactive oxygen species (ROS). Free radicals, components of ROS are found in cigarette mainstream and side stream smoke. Side stream Cigarette smoke contains more toxic gases and free radicals than the mainstream cigarette smoke (Church *and* Pryor, 1985). The adverse effects of smoking may result from the accumulation of oxidative damage bought about by ROS, which is called oxidative stress (Avogbe, et al. 2005).

To prevent further impact of cigarette smoke on health, the provision of intake of antioxidant compounds can be an effective therapeutic alternative for smokers. According to Halliwell and Gutteridge (1992), antioxidants can act as a catcher (scavenger) so that the free radical chain oxidation reaction can be interrupted. One of the antioxidant is mangosteen pericarp extract. Crude ethanol extract of mangosteen pericarp (CEMP) contains high concentration of antioxidants. The main natural antioxidants in CEMP are polyphenols, and one of the polyphenols is the flavonoid.

Treatment crude ethanol extract of mangosteen pericarp (CEMP) on rats that exposed by cigarette hopefully can improve the profile of bloods (MDA) level and the lung of rats.

1.2 Problem statement

1. How is the result of phytochemical screening from CEMP?
2. Is CEMP can reduce malondialdehyde (MDA) levels on the rats that exposure by cigarette?
3. Is CEMP can repair the lung histology profiles on the rats that exposure by cigarette?

1.3 Objectives

1. To prove the CEMP characteristics based on the phytochemical screening.
2. To prove the MDA-lowering potency of CEMP on the rats that exposure by cigarette.
3. To prove that CEMP has a potency to repair the lung histology profiles on the rats that exposure by cigarette.

II. LITERATURE REVIEW

2.1 Mangosteen (*Garcinia mangostana Linn*)

The mangosteen (*Garcinia mangostana Linn*) tree has been cultivated for centuries in tropical countries such as Indonesia, Malaysia, Myanmar, Thailand, Cambodia, Vietnam and rhe Moluscas (Akao et al. 2008). This tree can reach 6 - 25 m and it has leathery, glabrous leaves and is slow to grow (Morton, 1987).

The mangosteen-fruit is dark purple or reddish, with white, soft and juice edible pulp with a slightly acid and sweet flavor and pleasant aroma. The entire fruit of mangosteen is typically 2.5-7.5 cm in diameter, roughly the size of a tangerine (Figure 1B). The rind (or skin) of the fruit is 0.6-1.0 cm thick and contains a purplish pigment. The inner pulp consists of four to eight juicy, white-colored segments (fruit portion, Figure 1B). The edible portion of the fruit comprises only about 25% of the total volume, whereas the remainder is tough, bitter pericarp which exudes a yellow resin (hence the term xanthones or yellow in Greek) (Figure 1B). The mangosteen rind, leaves and bark have been used as folk medicine for thousands of years. The thick mangosteen rind has been and is used for treating catarrh, cystitis, diarrhea, dysentery, eczema, fever, intestinal ailments, pruritis and other skin ailments. The mangosteen leaves are also used by some natives in teas and for diarrhea, dysentery, fever, and thrush. It is also known that concentrates of mangosteen bark can be used for genito-urinary afflictions and stomatosis (Akao et al. 2008).

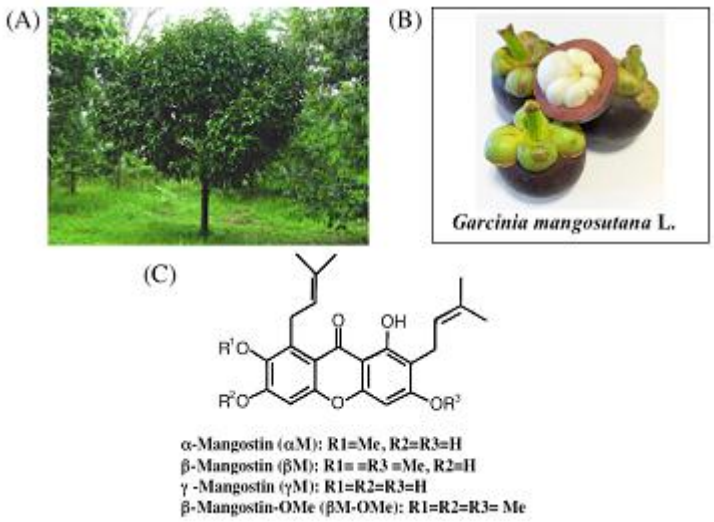

Figure 1. The *Garcinia mangostana* Linn tree (A), the appearance of mangosteen fruit (B) and the chemical structures of xanthones included in the pericarps (C) (Akao et al. 2008).

2.2 Xanthones

The xanthones possess a six-carbon conjugated ring structure with multiple double carbon bonds. The chemical structures of 4 major xanthones contained in percarps are shown in Figure 1C. The prenyl group is considered to be implicated in the internalization into the cell, which in turn leads to interaction with the signal transduction molecules and the proteins involved in mitochondria permeability transition (Watjen, et al.2007; Bae et al. 2006).

Xanthones or xanthen-9H-ones are secondary metabolites found in some higher plant families, fungi and lichens (Peres et al., 2000; Vieira and Kijjoa, 2005), and they comprise an important class of oxygenated heterocycles. The xanthone nucleus is known as 9-xanthenone or dibenzoc-pyrone and it is symmetric (Fig. 1) (Vieira and Kijjoa, 2005; Pinto et al., 2005; Souza and Pinto, 2005; Gales and Damas, 2005). Xanthones have been classified in five groups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated

xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Sultanbawa, 1980; Jiang et al., 2004).

2.2.1 Xanthones isolated from the pericarp of mangosteen-fruit

Fifty xanthones have been isolated from pericarp mangosteenfruit. The first of them was named mangostin (after it was named a-mangostin) when it was isolated in 1855 (Fig. 1) (Schmid, 1855). It is a yellow coloring matter that can also be obtained from bark and dried sap of GML (Dragendorff, 1930).

Later, Dragendorff (1930) and Murakami (1932) elucidated the mangostin structure. Yates and Stout (1958) established the molecular formula, and type and position of substituents of α-mangostin. Furthermore, Dragendorff (1930) isolated b-mangostin, the structure of which was not elucidated until 1968. Jefferson (1970) and Govindachari and Muthukumaraswamy (1971) also isolated α- and β-mangostins.

Recently, mangosharin was isolated from the bark of GML (Ee et al., 2006) and α- and β-mangostins were isolated from the root of Cratoxylum cochinchinense, which is a shrub tree belonging to the Guttiferae family (Laphookhieo et al., 2006).

Other xanthones that have been isolated from the pericarp of mangosteen-fruit are c-mangostin (Jefferson et al., 1970), gartanin and 8-deoxygartanin (Govindachari and Muthukumaraswamy, 1971), 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-isopre- nyl-2H,6H-pyrano [3,2-b] xanthen-6-one (Sen et al., 1980a), garcinone A, B and C (Sen et al., 1980a, 1982), garcinone D (Sen et al., 1986), garcinone E (Dutta et al., 1987), BR-xanthone A and BR-xanthone B (Balasubramanian and Rajagopalan, 1988), 1,5-dihydroxy2-isoprenyl-3-methoxyxanthone, 1,7-dihydroxy-2-isoprenyl3-methoxy xanthone and mangostinone (Asai et al., 1995), 2,7-di-isoprenyl-1,3,8-trihydroxy-4-methyl

xanthone and 2,8-diisoprenyl-7-carboxy-1,3,-trihydroxy-4-methyl xanthone (Gopalakrishnan and Balaganesan, 2000), mangostanol (Chairungsrilerd, 1996a), euxanthone (Gopalakrishnan et al., 1997), garcimangosones A, B, C and D, tovophyllin A and B and 1,3,6,7-tetrahydroxy-8-isoprenyl-9H-xanthen-9-one (Huang et al., 2001), mangostenol, mangostenone A and B (Suksamrarn et al., 2002), 2-isoprenyl-1,7-dihydroxy-3-methoxyxanthone (Matsumoto et al., 2003), compound 7 and mangostanine (Suksamrarn et al., 2003), 8-hydroxycudraxanthone G, mangostinone and esmeatxanthone A (Jung et al., 2006), caloxanthone A, macluraxanthone and 1,7-dihydroxyxanthone (Iinuma et al., 1996). Smeathxanthone A has also been isolated from Garcinia smeathmannii (Komguem et al., 2005).

Calabaxanthone was isolated from the bark of Calophyllum calaba and Calophyllum bracteautum in 1972 (Somanathan and Sultanbawa, 1972), it was studied by ^{13}C MNR (Westerman et al., 1977) and later was also isolated from the pericarp of mangosteen-fruit (Mahabusarakam et al., 1987; Sen et al., 1980).

Seven new xanthones were isolated from the pericarp of mangosteen-fruit in 1987: 1-isomangostin, 1-isomangostin hydrate, 3-isomangostin and 3-isomangostin hydrate (Mahabusarakam et al., 1987). 2-(c,c-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone, demethylcalabaxanthone, 1,3,7-trihydroxy-2,8-di(3-methylbut-2-enyl)xanthone and 2,8 bis (c,c-dimethylallyl)-1, 3,7-trihydroxyxanthone were isolated of the arils (seed coats). They also obtained several xanthones already isolated (mangostin, gartanin, b-mangostin, c-mangostin, and calabaxanthone).

Recently, a- and b-mangostin, 9-hydroxycalabaxanthone, 3isomangostin, gartanin, and 8-desoxygartanin have been extracted of the fruit rind of mangosteen, identified and quantitatively determined used high performance liquid chromatograpy (HPLC) (Walker, 2007). The xanthones 3-isomangostin, 8-

desoxygartanin, gartanin, a- and b-mangostins and 9-hydroxycalabaxanthone also have been identified by UV spectra and quantified by HPLC with photodiode array detector and HPLC with time-of-flight mass spectrometry system coupled with electrosplay ionization interface (Ji et al., 2007).

2.3 Main biological and medicinal properties of Mangosteen

2.3.1 Antioxidant properties

Weecharangsan et al. (2006) studied the antioxidant and neuroprotective properties of four extracts obtained from mangosteenfruit pericarp (water, 50% ethanol, 95% ethanol and ethyl acetate). The antioxidant capacity was evaluated by the DPPH method using 1, 10, 50 and 100 lg/mL of each extract. Water and ethanolic (50%) extracts showed high antioxidant capacity (inhibitory concentration at 50% (IC_{50}) = 34.98 ± 2.24 and 30.76 ± 1.66 µg/mL, respectively). The antioxidant capacity of these extracts was tested on a neuroblastoma cell line (NG108-15) exposed to hydrogen peroxide (H2O2); both extracts exhibited neuroprotective activity when they used concentration of 50 µg/mL. The 50% ethanolic extract had higher neuroprotective activity than the water extract. More recently, Chomnawang et al. (2007) showed that GML ethanolic extract possesses a significant antioxidant activity, as measured by the inhibition of the formation of DPPH radicals by 50%. This extract displayed an IC_{50} of 6.13 µg/mL in comparison with ethanolic extracts of Houttuynia cordata, Eupatorium odoratum and Senna alata (IC_{50} of 32.53, 67.55 and 112.46 µg/mL, respectively). In addition, the extract of G. Mangostana significantly reduced the reactive oxygen species (ROS) production of polymorphonuclear leucocytes (PML) with 77.8% of superoxide anion (O_2^-) inhibition ratio (62.6%, 44.9% and 35.18% for H. cordata, E. odoratum, and S. alata, respectively). Haruenkit et al. (2007) showed the antioxidant activity of mangosteen measured with DPPH and ABTS assays.

They found values of 79.1 and 1268.6 µM trolox equivalents/ 100 g of fresh weight for DPPH and ABTS assays, respectively. In addition, in rats fed with basal diet supplemented with 1% of cholesterol plus 5% of mangosteen the increase in plasma lipids and decrease in antioxidant activity seen with cholesterol alone was prevented. Devi Sampath and Vijayaraghavan (2007) evaluated the effect of α-mangostin on the antioxidant defense system and on lipid peroxidation during isoproterenol-induced myocardial infarction in rats. Treatments of rats with isoproterenol (150 mg/kg for 2 days) showed a significant decrease of the antioxidant enzymes glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH); as well as marked elevation in serum enzymes such as lactate dehydrogenase (LDH), creatine phosphokinase (CPK), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lipid peroxides. The histological examination of rats treated with isoproterenol showed necrotic changes in the tissue with intense infiltration of neutrophils. Pretreatment with a-mangostin (200 mg/kg) for 6 days prior and 2 days concurrently with isoproterenol administration significantly attenuated these changes. This xanthone showed a protective effect against lipid peroxidation and antioxidant defense system during injury-induced myocardial infarction in rats.

2.3.2 Anti-tumoral Properties

Ho et al. (2002) found that garcinone E has a potent cytotoxic effect on hepatocellular carcinoma cell lines. They studied the cytotoxic effect of 6 xanthones isolated from mangosteen-fruit pericarp and found that garcinone E was the most toxic. Therefore, garcinone E was tested against HCC36, TONG, HA22T, Hep3B, HEpG2 and SK-Hep-1 hepatocellular carcinoma cell lines; NCIHut 125, CH27 LC-1, H2891 and Calu-1 lung carcinoma cell lines; and AZ521,

NUGC-3, KATO-III and AGS gastric carcinoma cell lines. Garcinone E exhibited a very broad spectrum of dose- and timedependent cytotoxic effects against various cancer cell lines; with the exception of lung carcinoma cell line CH27 LC-1, all cell lines tested were killed. The values for garcinone lethal dose 50% (LD_{50}) against the cell lines studied were between 0.1 and 5.4 µM. Garcinone E had an antitumoral effect in the following order: SK-Hep-1 > HA22T > HEpG2 > Hep3B > HCC36.

Suksamrarn et al. (2006) isolated from mangosteen-fruit pericarp three new prenylated xanthones (mangostenones C, D and E) as well as 16 known xanthones. The cytotoxic properties of these xanthones were determined against three different human cancer cell lines: epidermoid carcinoma of mouth (KB), breast cancer (BC-1), and small cell lung cancer (NCI-H187). Mangostenone C exhibited a cytotoxic effect against the three cell lines proved, with IC_{50} values of 2.8, 3.53, and 3.72 lg/mL, respectively. However, amangostin exhibited the most potent effect against BC-1 cells with an IC50 value of 0.92 µg/mL, an activity that was greater than the standard drug ellipticine (IC50 = 1.46 lg/mL); α-mangostin also had a cytotoxic effect against KB cells (IC_{50} = 2.08 µg/mL; and gartanin was able to inhibit the NCI-H187 growth (IC_{50} = 1.08 µg/mL. In summary suggest that α-mangostin and its analogs would be candidates for preventive and therapeutic application for cancer treatment.

2.3.3 Anti-inflammatory and anti-allergy properties

Chairungsrilerd et al. (1996c) demonstrated that methanolic extract of mangosteen-fruit pericarp inhibits the contractions of isolated thoracic rabbit aorta induced by histamine and serotonin. They suggested that α- and γ-mangostins are histaminergic and serotonergic receptor blocking agents, respectively. This same research group studied the effect of a-mangostin on histamine-induced

contractions in rabbit thoracic aorta and guinea-pig trachea (Chairungsrilerd et al., 1996a). α-mangostin inhibited histamine-induced contractions in a dose-dependent manner with or without cimetidine, an antagonist of the H_2-histamine receptor. Also, α-mangostin inhibited contractions mediated by the histamine H_1 receptor. Furthermore, α-mangostin competitively inhibits [^{3}H] mepyramine (specific antagonist of histamine H_1 receptor) binding to rat aortic smooth muscle cells.

The anti-inflammatory effects of α- and γ-mangostins were evaluated by carrageenan-induced paw edema in mice. The α-mangostin and sulindac (reference compound) treatment showed a potent inhibition of paw edema at 3 h and 5 h, respectively. The action of α-mangostin was more rapid than that of sulindac. However, γ-mangostin did not significant inhibit the paw oedema in mice. This demonstrated that in vivo α-mangostin has more anti-inflammatory activity than γ-mangostin. In addition, Deschamps et al. (2007) demonstrated that α-mangostin inhibited 12-human lipoxygenase (12-LOX) with an IC_{50} of 0.58 μM.

The IgE receptor activates intracellular signal transductions resulting in the release of inflammatory signal mediators such as histamine and this is the primary event in several allergic responses. Based on this information, Itoh et al. (2008) demonstrated that xanthones isolated from mangosteen (α, β and γ-mangostins) suppressed the degranulation in Ag-mediated activation of IgE receptors in rat basophilic leukemia RBL-2H3 cells. These authors suggest that the inhibitory mechanism of degranulation by xanthones was mainly due to suppression of the SYK/PLC cs/PKC pathway. The research above indicated that xanthones isolated from mangosteen could be a novel target of anti-inflammatory and antiallergic compounds.

2.3.4 Antibacterial, antifungal and antiviral properties

Sundaram et al. (1983) studied the antibacterial and antifungal properties of a-mangostin and four of its derivatives. They found that bacteria *S. aureus, P. aeruginosa, Salmonella typhimurium* and *Bacillus subtilis* were highly susceptible to xanthones, whereas *Proteus sp., Klebsiella sp*. and *Escherichia coli* were only moderately susceptible to them. About fungi, *Epidermophyton floccosum, Alternaria solani, Mucor sp., Rhizupus sp.* and *Cunninghamella echinulata* were also highly susceptible to xanthones, whereas *Trichophyton mentagrophytes, Microsporum canis, Aspergillus niger, Aspergillus flavus, Penicillium sp., Fusarium roseum* and *Curvularia lunata* were only moderately susceptible to them. The minimum inhibitory concentration (MIC, the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation) of α-mangostin was between 12.5 and 50 µg/mL for bacteria and between 1 and 5 µg/mL for fungi. The order of the antibacterial and antifungal efficiency was as follows: a-mangostin > isomangostin > 3-O-methyl mangostin > 3,6-di-O-methyl mangostin. Mangostin triacetate had no activity.

Gopalakrishnan et al. (1997) demonstrated the antifungal activity of several xanthones isolated from mangosteen-fruit pericarp and some α-mangostin-derivatives against three phytopathogenic fungi (*Fusarium oxysporum vasinfectum, Alternaria tenuis* and *Dreschlera oryzae*). α-mangostin, γ-mangostin, gartanin, garcinone D, BR-xanthone and euxanthone showed high inhibitory activity against the three fungi; they used 1, 10, 100 and 1000 ppm in the culture medium. Substitution in A and C rings has been shown to modify the bioactivities of the compounds.

Several natural products have been identified because of their capacity to inhibit different stages in the replication cycle of human immunodeficiency virus (HIV-1). Among them, xanthones have been shown to inhibit proteolytic cleavage

by protease inhibition (reviewed in Vlietinck et al., 1998). Chen et al. (1996) showed that ethanolic extract of GML effectively inhibited HIV-1 protease. Two xanthones were isolated from the ethanolic extract: a- and c-mangostins, which exhibited an IC_{50} value of 5.12 ± 0.41 and 4.81 ± 0.32 µM, respectively. Pepstatin A (IC_{50} = 76 ± 5.5 nM) was used as positive control.

2.3.5 Antimalarial properties

Several xanthones isolated from mangosteen have shown antimalaria activity in vitro against *Plasmodium falciparum.* β-mangostin and α-mangostin exhibited a comparable IC_{50} value (7 and 5.1 µM respectively), whereas mangiferina, a xanthone-glucoside, exhibited an IC_{50} value higher than 50 µM(Riscoe et al., 2005). In the other hand, Mahabusarakam et al. (2006) found that a-mangostin exhibited an IC50 value of 17 µM against *P. falciparum*. Laphookhieo et al. (2006) found that β-mangostin isolated from roots of C. cochinchinense had an IC50value of 7.2 µg/mL against *P. falciparum.*

2.4 Hazard of smoking

Smoking caused many dangerous diseases like lung cancer, tumors and others. Data from the Disease & Disorder Company, USA, mentioned that there are about 200 types of content of toxins in cigarettes. One's chances of getting cancer in smokers was 1.6 times higher than people who do not smoke (Main, 2004).

According to WHO, smoking has been taking part in 90% of cases of lung cancer in men and 80% in women (Dams et al, 1998). Harmful substances in cigarettes that cause harmful diseases in humans there are various types, among the most commonly known effect is a kind of tar, nicotine, carbon mono oxide,

ammonia, nitrosamines, hydrogen cyanide, cyanogen, peroxides, oxidant compounds of sulfur, nitrogen oxides, aldehydes, and ketones (Kompas, 2003). Among the chemicals that are most associated with the presence of various diseases caused by smoking are (1) Tar containing toxic chemicals damaging lung cells and cause cancer, (2) Nicotine is a stimulant drug that can damage the heart and blood circulation (Dysfunction endothelial), and can be addictive, and (3) CO (Karbonmonooksida) which is a toxic gas which causes a decrease in the ability of oxygen-carrying red blood grains. In the end, all the harmful ingredients of cigarettes will stimulate excessive production of free radicals or oxidants in the human body (Murray, 1996).

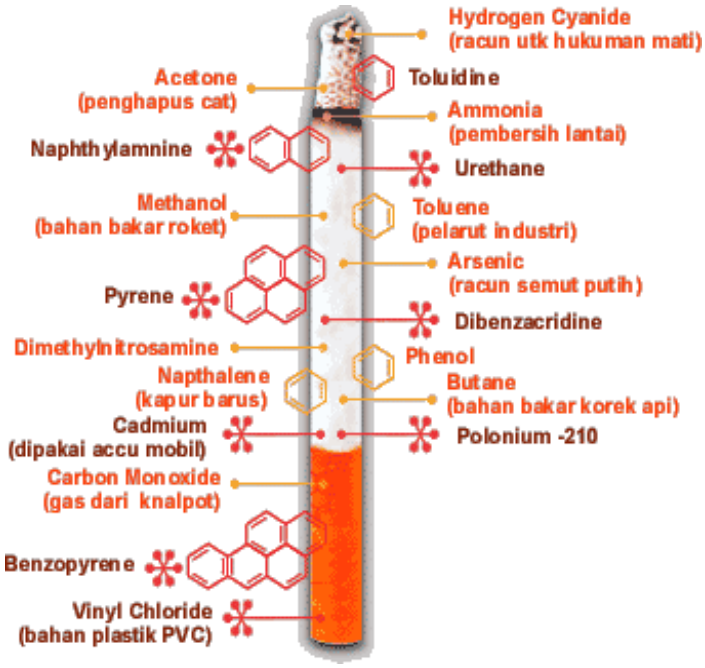

Figure 2. Cigarettes and toxic compounds

Incomplete combustion of cigarette smoke will be generated main (Main Stream Smoke) and the smoke side (Side Stream Smoke or Secondhand Smoke).Smoke is a major part of tobacco smoke directly inhaled by active smokers and have been filtered by a filter cigarette. And smoke next to an

understanding as a result of the burning end of tobacco smoke are not perfect and can be inhaled by passive smokers or smoke released from the lungs back to active smokers. Smoke is a very big side effects to health, because there are quite a lot and levels of hazardous materials they contain are still quite high, thus allowing the emergence of various diseases that are not desired on and off (Gatra, 2000).

III. PROJECT OUTLINE

3.1 Research Framework

Mangosteen, Garcinia mangostana Linn, is a tree found in South East Asia and its pericarps have been used as traditional medicine. Among the constituents of mangosteen peel, xanthones are biologically active phenols that naturally occur in restricted group of plants (Suksamrarn et al., 2006). Over 200 xanthones are currently known to exist in nature and approximately 50 of them are found in the mangosteen (Watjen, et al.2007; Bae et al. 2006). The 4 structurally similar xanthones are α, β, ϒ and methoxy- β-mangostin. The compound of α, β and ϒ mangostin turns out have biological activity such as antioxidant (Weecharangsan et al. (2006), anticancer, antitumor, antiinflammatory (Suksamrarn et al., 2006; Ho et al., 2002; Chairungsrilerd et al., 1996c), antibacterial, antituberculosis (Suksamrarn, 2003), inhibited cell growth in human colon cancer (Akao e*t al*. 2008). Moreover, α-mangostin was found to have a cancer preventive effect in rats carcinogenesis bioassay and the extract from peel, which contains mainly α-mangostin and ϒ-mangostin, exhibited an enhancement of NK cell activity in a mouse model (Nakatani *et al*. 2004).

Smoking caused many dangerous diseases like lung cancer, tumors and others. Data from the Disease & Disorder Company, USA, mentioned that there are about 200 types of content of toxins in cigarettes. One chances of getting cancer in smokers was 1.6 times higher than people who do not smoke (Main, 2004). Treatment ethanol extract of mangosteen pericarp (CEMP) on rats that exposure by cigarette hopefully can improve the profile of bloods, and lung of rats.

3.2 Research Concept

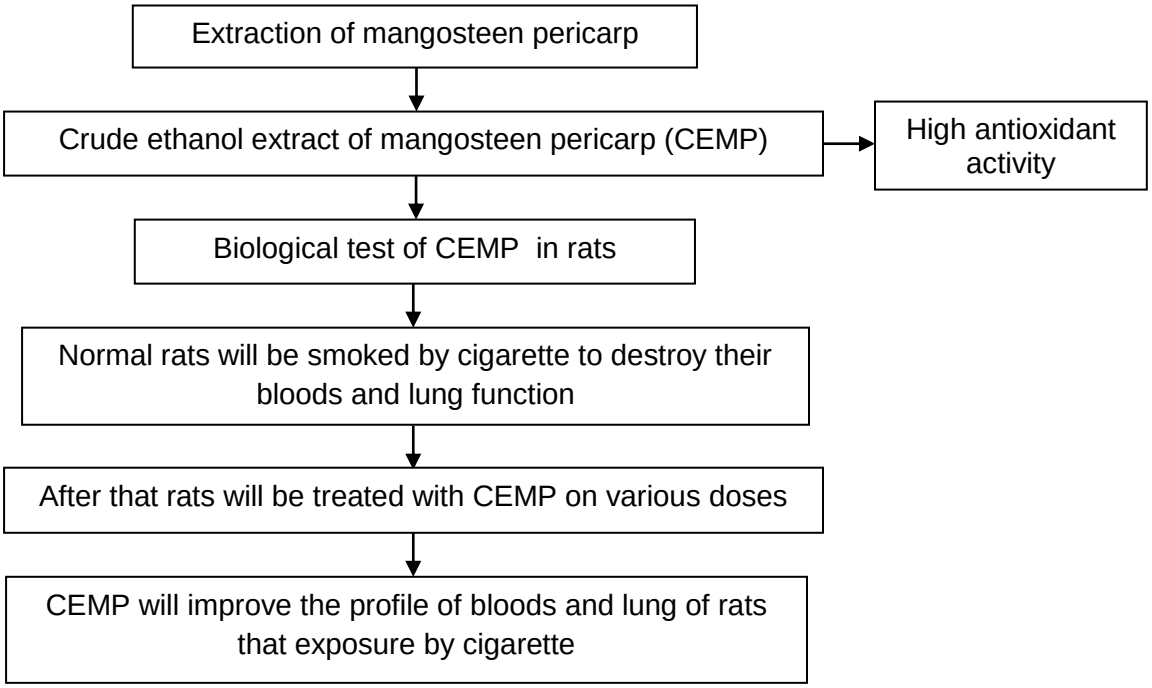

Figure 3. Research concept

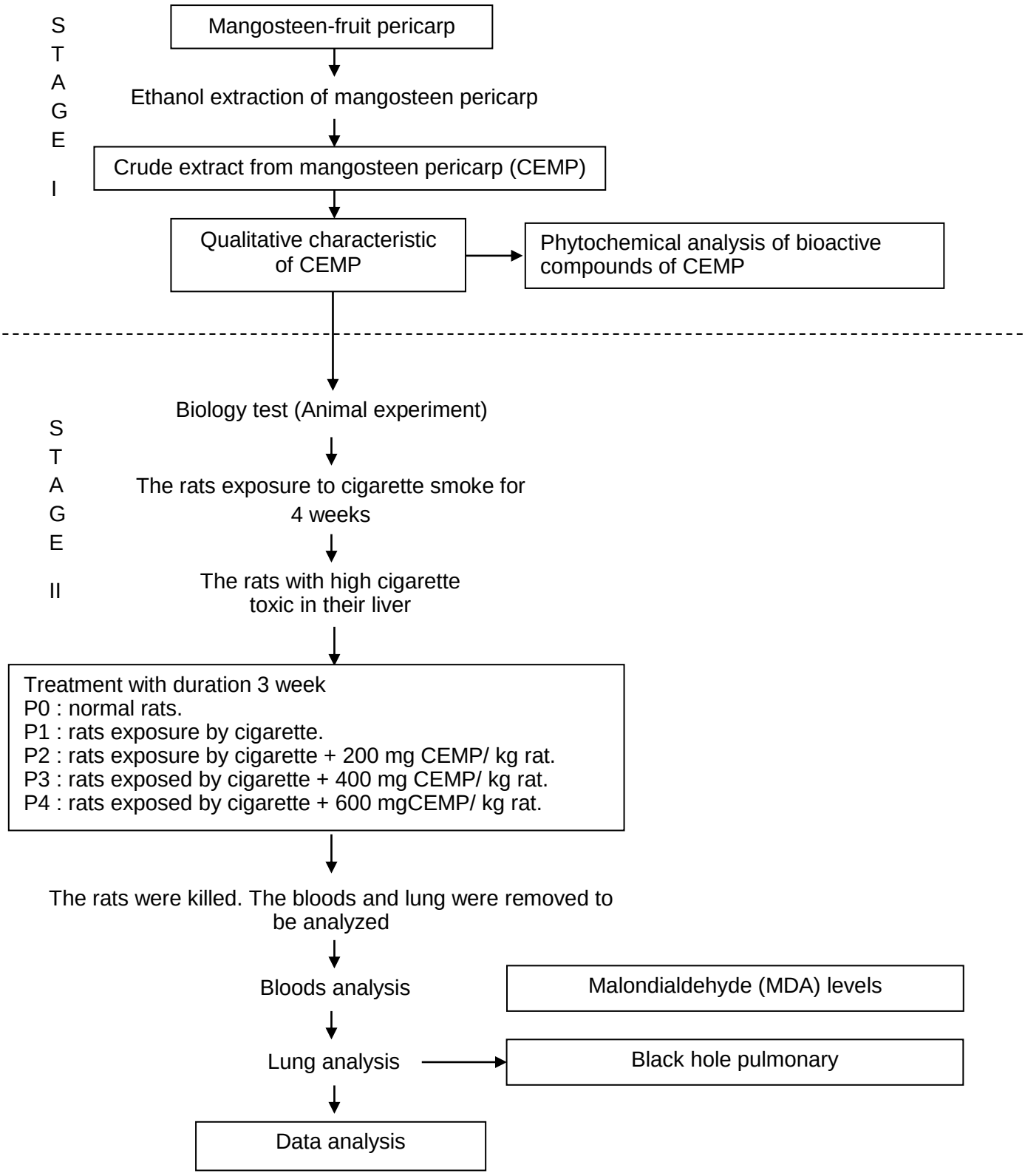

Figure 4. Operational Concept

3.3 Hypothesis

Hypothesis of these research are:

1. CEMP contain high antioxidant compounds.
2. CEMP can reduce MDA levels of the rats that exposure by cigarette.
3. CEMP can decrease the black hole pulmonary in the lung of the rats that exposure by cigarette.

3.4 Research Variable

1. Independent variable : doses of the crude ethanol extract of pericarp mangosteen (CEMP) in rats.
2. Dependent variable : MDA levels, and histology profiles of the lung.

IV. RESEARCH METHODOLOGY

4.1 Experimental Design

This study was conducted in a completely randomized post-test only design that compared five groups of rats:

P0: (negative control) Normal mice without exposure to cigarette smoke

P1 : (positive control) Rats that were exposed to cigarette smoke

P2: Rats that were exposed to cigarette smoke giving CEMP + 200 mg / kg rat

P3: Rats that were exposed to cigarette smoke giving CEMP + 400 mg / kg rat

P4: Rats that were exposed to cigarette smoke giving CEMP + 600 mg / kg rat

The experiments was carried out for two months in an animal house at animal physiology laboratory, Faculty of Science and Mathematics, Brawijaya University, Malang.

4.2 Crude ethanol extract from mangosteen pericarp (CEMP)

The whole pericarp (outer and inner peels) of G. mangostana were harvested from Malang, East Java, Indonesia. The samples were first cleaned to remove any residual compost and washed thoroughly to remove impurities. After washing, the samples were chopped into small pieces (0.5 x 1.0 cm^2) and dried over a night in a tray dryer at 45^oC. then chopped samples were ground with a grinder to make powder (around 18 mesh).

All ground samples were placed in 70^oC distilled water for 15 min at the ratio of sample powder : water of 1:4. The mixtures were boiled 4 times or until no content of tannin was found by dropping 2% gelatin solution in the mixtures (Weecharangsan et al. 2006). The mixtures were filtrated, the residues were then dried at 40 -45^oC in the hot air oven. The dried powder was macerated at room temperature for 7 days with 50% ethanol. The crude extract were filtered and evaporated at 40 -45^oC in the hot air oven to obtain the dried crude extracts. The obtained extracts were stored in a desicator containing dry silica gel prior using in each experiment.

4.3 Protocol for preliminary phytochemical screening

The test have been done to find the presence of the active chemical constituents such alkaloids, steroids, flavonoids by the following procedure.

4.3.1 Alkaloids

Alkaloids solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (Siddiqui and Ali,1997).

4.3.2 Steroids

20 mg of extract was treated with 2.5 ml of acetic anhydride and 2. 5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids (Siddiqui and Ali, 1997).

4.3.3 Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange colour for flavones (Siddiqui and Ali, 1997).

4.3.4 Terpenoids

2 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. a greylish colour indicates in the presence of the terpenoids (Njoku and Obi, 2009).

4.3.4 TLC analysis of the fraction

Extract was checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel (TLC-grade, Merck). This eluent is diethyl ether: ethyl acetate: acid acetate 3 : 4 : 4. The lack of the study is not use standard for solvent.

4.4 Doses determination

Based on the results from an acute and repeated dose-toxicity study, it was conclude that for single oral dosage up to 5 mg/kg body weight mangosteen pericarp extract and for continuous oral administration (28 days), up to 1000 mg/kg body weight did not cause any significant toxic effect in rats (Pathom et al. 1998). Oral and intraperitoneal administration (50 mg/kg) of α-mangostin, 1-isomangostin and mangostin triacetate exhibited anti-inflammatory activity in rats tested by the carrageenan-induced hind paw edema (M, 1M and MT showed 66.6%, 63.19% and 59.03%) (Shankaranarayan *et al*. 1979). So in this research we use CEMP doses: P3: 200 mg/kg; P4: 400 mg/kg and P5:600 mg/kg body weight of rats.

4.5 Animals and diets

Thirty male Wistar weighing between 180 and 200 g were purchased from from animal physiology laboratory, Science and Mathematics Faculty, Brawijaya University, Indonesia. Estimated number of repetitions or the size of the sample in this study can be calculated using the formula:

$$(n-1)\,(p-1) \geq 15$$

$$(n-1)\,(5-1) \geq 15$$

$$n-1 \geq 15/4$$

$$n-1 \geq 3.75$$

$$n \geq 4.75$$

Description: n = number of repetition for each treatment group

p = number of treatment groups

So, in this research used 5 repetition for each group, and the overall number of samples used 25 rats.

Twenty five male Wistar rats with average body weight of 180 - 200 g were used in this experiment. These rats were divided into five groups (P0, P1, P2, P3 and P4) that consisted of 5 rats each. These rats were individually kept in a plastic cage in a temperature-controlled room (22 ± 2^{o}C) under a light/dark cycle of 12 h. Groups P1, P2, P3 and P4 were exposed by cigarette for 4 weeks. Once the production of reactive oxygen species (ROS) was reached, the rats received CEMP treatment. Group P2, P3 and P4 received CEMP (200, 400 and 600 mg/ kg weighing rats) by gastric intubations every day during 3 weeks together with exposed cigarette. Control CE animals (P1) are exposed cigarette without CEMP. And control normal animals (P1) are no exposed by cigarette and without CEMP. Blood was collected by intra vena of rat tail after treatment by CEMP. Blood was centrifuged for 15 min at 3500 rpm. Plasma was used to determination of MDA levels. At the end of the experimental period, the rats were killed by decapitation. The lung was removed and rinsed with physiological saline and was stored at -80^{o}C until be analyzed.

4.6 Malondialdehyde (MDA) measurements

Measurement of MDA level was done according to the method of Soewoto et al. (2001) in brief: the blood sample was added to 0.50 mL of 10% cold TCA solution, which was then centrifuged for 15 minutes. The supernatant formed was added to 0.75 mL of 0.67% TCA solution, and the mixture was then placed into a boiling-water containing water bath for 10 minutes. After it was cold, it was read using a spectrophotometer at a wavelength of 532 nm to determine the MDA concentration.

4.7 Histopathological analysis of black hole pulmonary in the lung of rats

The excised largest lobe of left lung was inflated with 1 ml formalin under constant pressure before fixing with formalin, then dehydrated with ethanol, and embedded in paraffin. The lung lobe was cut at 5 µm. Sections were stained with hematoxylin and eosin

for histologic examination. Images of 10 fields for each lung section were captured randomly at x40 magnification. Each image was then analyzed using AxioVision (Ziess, Germany). Alcian blue (AB)-periodic acid-Schiff (PAS) staining was used to examine goblet cell hyperplasia. Images of 3 fields for epithelium in cartilaginous bronchus were captured randomly at x100 magnification (Chan et al. 2009).

4.8 Data analysis

Results of MDA are expressed as mean ± standard error of the mean (S.E.M) and for histophatological by descriptive. To determine the effect of CEMP on the MDA levels, data were analysed by one way analysis of variance (ANOVA) using the general linier model procedure of SPSS 16.0 software. Significant differences between dietary treatments were analysed by Post-Hoc Tukey. $P < 0.05$ values were considered as significantly different.

V. RESULTS AND DISCUSSION

5.1 Phytochemical Screening

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for mangosteen pericarp extract showed significant indication about the presence of metabolites. Flavonoids, terpenoids and alkaloids were found to be present in the extract of the pericarp. These findings of phytochemicals were good enough to reflect its importance.

Table 1. Qualitative phytochemical properties of CEMP by phytochemical analysis

Phytochemicals	Pericarp extract of mangosteen
Flavonoid	+
Terpenoid	+
Alkaloid	+

(+) presence.

The phytochemical analysis showed that the CEMP contained bioactive compounds as , Flavanoids , Terpenoids and Alkaloids (Table 1)

5.2 TLC profiling of CEMP

After phytochemical analysis, the TLC result showed the 3 spots, namely A, B and C (Fig. 5) by UV confirmation. The 3 spots have different Rf value (Table 2).

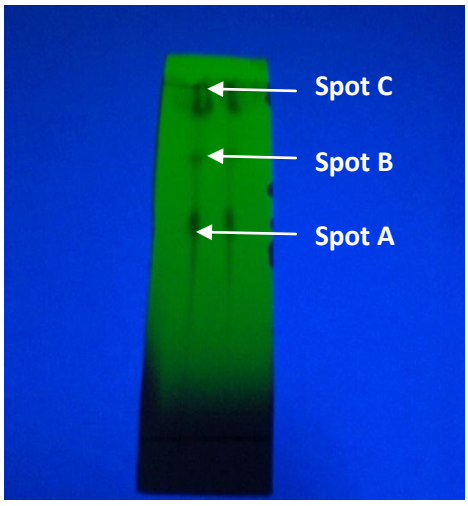

Figure 5. TLC result from mangosteen pericarp extract uses eluent diethyl ether : ethyl acetate : acid acetate 3 : 4 : 4

TLC profiling of pericarp extract gives an impressive result that directing towards the presence number of phytochemical analysis. Rf value is the height or length the component travelled or eluted from the starting point divided by the total length the mobile phase or solvent travelled, mainly used in TLC (thin layer chromatography), The variation of Rf influenced by (1) chemistry structure from the compounds that will be fractionation, (2) absorbent characteristic and it's activities degree, (3) thickness and flatness of absorbent, (4) eluent purity or accuracy of eluent ratio if used in mixture, (5) saturation degree of container, and (6) temperature. Compound showing high Rf value in less polar eluent system have low polarity and with less Rf value have high polarity.

Table 2. The Rf value of each spot CEMP by TLC method

Spot	Distance of spot from the initial movement		Rf Value
	Spot	Eluent	
A	4,5	8	0,56
B	6,2	8	0,77
C	7,6	8	0,95

5.3 Spectra analysis of mangosteen pericarp extract by FTIR

To confirm the functional groups of the compound is used as the standard spectrum of gallic acid as standard that has the same basic framework with polyphenolic compounds. Gallic acid standard range has a spectrum with absorbance range between 426.89 cm^{-1} to 3554.50 cm^{-1}. Usually containing gallic acid functional groups just as OH, CH, C = C, and CO.

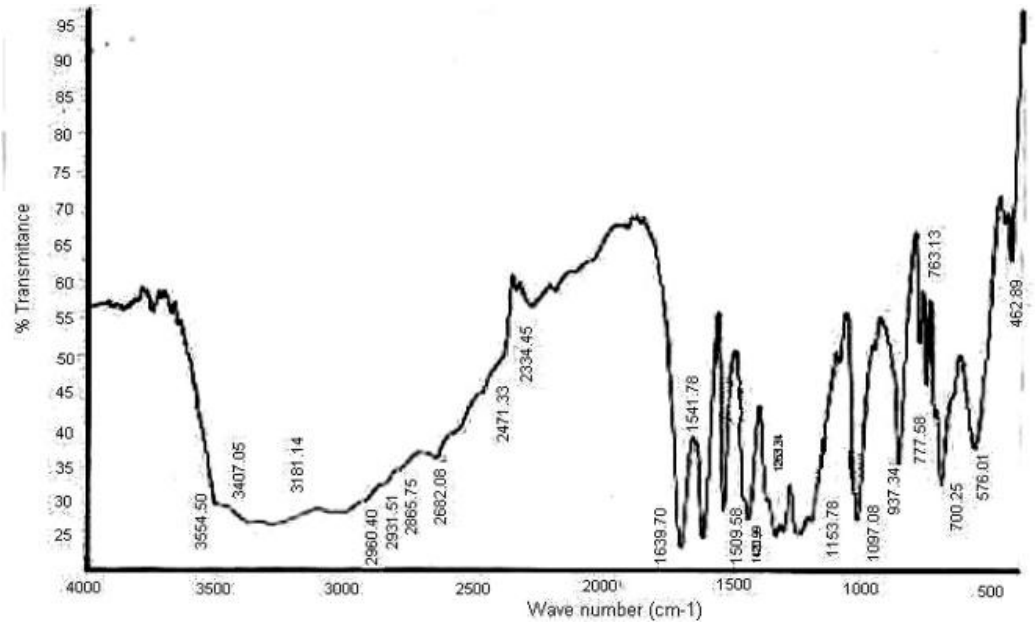

Figure 6. FTIR spectra of gallic acid standard

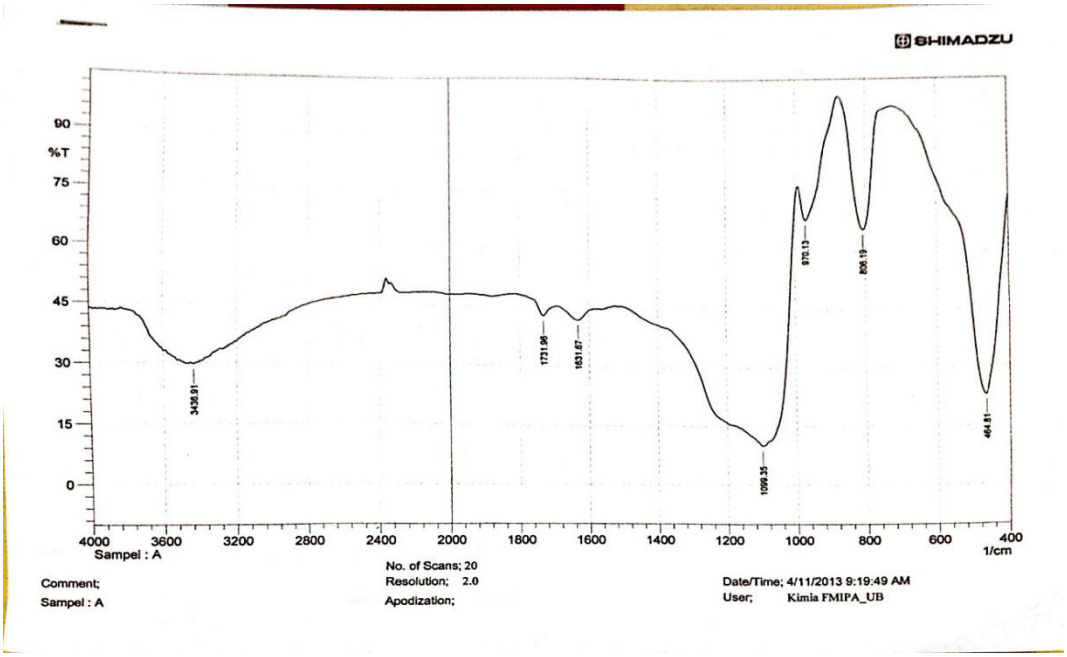

Figure 7. FTIR spectra of spot A TLC of CEMP

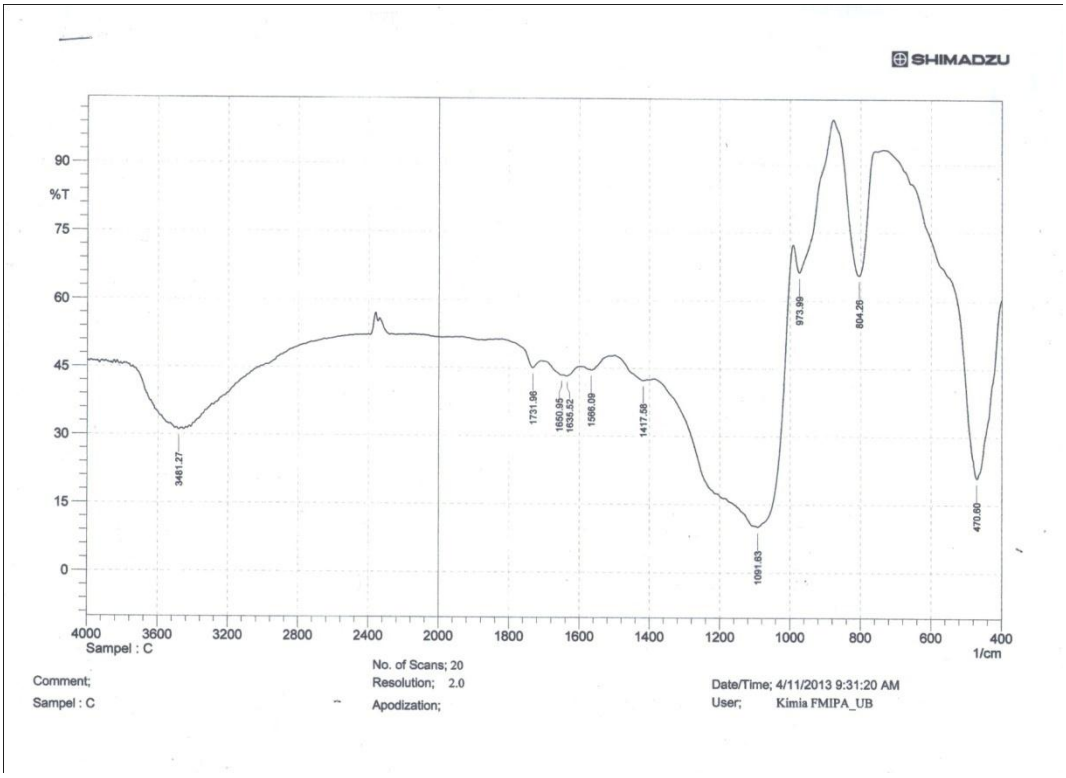

Figure 8. FTIR spectra of spot B TLC of CEMP

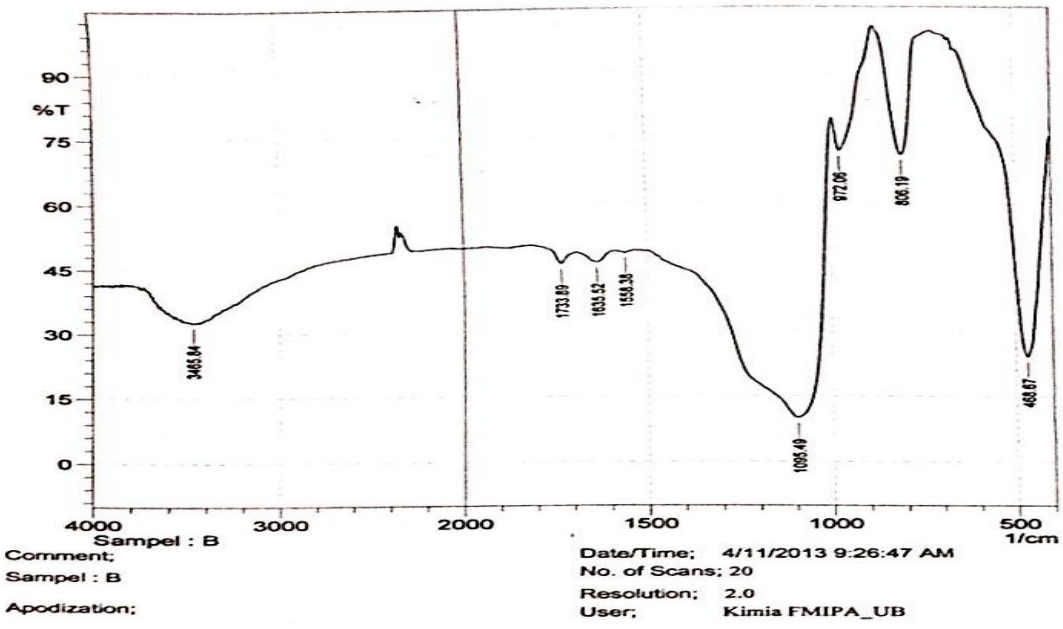

Figure 9. FTIR spectra of spot C TLC of CEMP

IR spectra of spot A shows the absorption at a wavelength of 3436.91 cm^{-1} which indicates the-OH group, 1731.96 cm^{-1} absorption indicates C = O aldehide, ketone, carboxylic acid and ester (Fig. 6). Absorption at 1631.67 cm^{-1} indicate the presence of C = C alkene, 1099.35 cm^{-1} indicated presence of CO (alcohol, ether, ester, and carboxylic acid) & CN amine, and at 806.19 cm^{-1} indicated the presence of CH aromatic. So it can be concluded that the A isolates contain several components such as the-OH, C = O, CO, C = C alkene and aromatic CH.

IR spectra of spot B (Fig. 8) shows the absorption at a wavelength of 3495.86 cm^{-1} indicating the presence of the-OH group, 1733.89 cm^{-1} absorption indicates C = O aldehide, ketone, carboxylic acid and ester. Absorption at 1635.52 cm^{-1} indicate the presence of C = C aromatic, 1558.38 cm^{-1} indicate the presence of aliphatic CH, 1095.49 cm^{-1} indicate the presence of CO. (alcohol, ether, ester, and carboxylic acid) & CN amine, and at 806.19 cm^{-1} indicate the presence of aromatic CH. So it can be concluded that B isolates contain several components such as the-OH, C = O, CO, C = C aromatic, aliphatic CH and aromatic CH.

IR spectra of C (Fig. 9) showed a spot absorption at a wavelength of 3481.27 cm^{-1} indicating the presence of the-OH group, 1731.96 cm^{-1} absorption indicates C = O aldehide, ketone, carboxylic acid and ester. Absorption at 1650.95 cm-1 indicate the presence of C = C alkene, 1635.52 cm^{-1} indicate the presence of aromatic C = C,, 1566.09 cm^{-1} indicate the presence of aliphatic CH. Absorption at 1417.58 and 1091.63 shows the CO (alcohol, ether, ester, and carboxylic acid) & CN amine and at 804.26 cm-1 indicate the presence of aromatic CH. So it can be concluded that the C isolate containing several components such as the-OH, C = O, aliphatic CH, CO, C = C alkenes, aromatic C = C and aromatic CH.

To confirm the presence of alkaloid class, use the standard spectrum of 4-piperidiniol because this compound is an alkaloid class of compounds that can be used as a standard for alkaloids alkaloid compounds may contain one or more nitrogen atoms. 4-piperidiniol. In Figure 8 and 9 indicates absorption at wave numbers between 612-3268 cm^{-1}. Alkaloid possessed functional groups generally are aliphatic CH and CN alkyl amines.

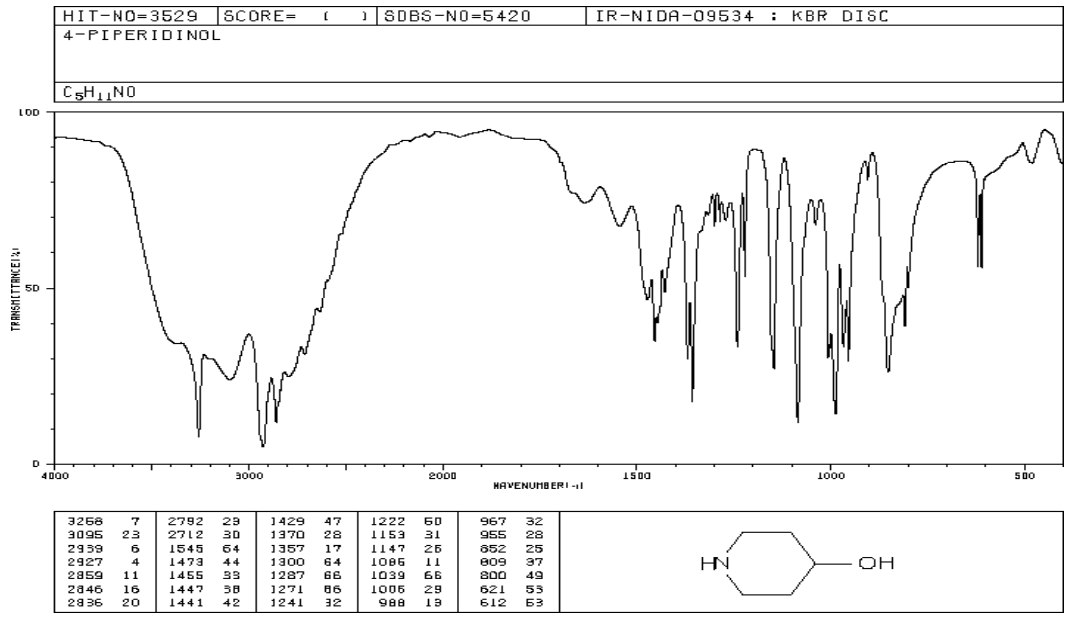

Figure. 10 FTIR spectra of 4-piperidiniol standard

Based on the results of the IR spectrum at three spots compared with a standard 4-piperidiniol spectrum is a class of alkaloid compounds. Aliphatic CH obtained in wave numbers 1558.38 and 1566.09, while the CN alkyl amines on the wave number 1099.35; 1095.49 and 1091.63.

Based on the IR spectrum has been obtained, it can be concluded that the compounds contained in an extract from the bark of the mangosteen contains components such as group-OH, C = C aromatic, C = C alkenes, aromatic CH, aliphatic CH, and CO group which also contained by group of compounds phenolik / polyphenols (flavonoids) and CN amines (alkaloids). IR spectra based stains B are compounds that have similar functional groups with standard gallic acid are polyphenolic compounds with a greater intensity of stain A stain that has a functional group OH, C = C alkene, C = C aromatic, aliphatic CH, CO and CH aromatic (Table 3).

Table 3. Interpretation of FTIR samples and standard

No.	Wavelength (cm^{-1})					Interpretation
	Spot A	**Spot B**	**Spot C**	**Gallic acid standard**	**Reference**	
1.	3436.91	3495.86	3481.27	3407.05 3554.50	3200-365	O-H (alcohol, phenol)
2.	-	-	-	2960.40 2931.51 2865.75	2850-3000	C-H aliphatic
3.	1731.96	1733.89	1731.96	-	1705-1750	C=O aldehide, ketone, carboxylic acid and ester
4.	1631.67		1650.95	1639.70	1600-1680	C=C alkynes
5.		1635.52	1636.52	1509.58	1475-1600	C=C aromatic
6.		1558.38	1566.09	1420.99	1370-1465	C-H aliphatic
7.	1099.35	1095.49	1417.58 1091.63	1153.78 1097.08	1000-1300 1020-1150	C-O (alcohol, eter, ester, and carboxylic acid) C-N amina
8.	806.19	806.19	804.26	777.58 763.13	690-900	C-H aromatic

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 3. The FTIR confirmed the presence of alcohols, phenols, aldehydes, ketone, carboxylic acid, ester, alkynes, aromatic, aliphatic, alcohol, ether, ester, and carboxylic acid, and amines in CEMP.

5.4 Invivo Study

Lung cancer is the most common cancer in the world and one of the most fatal cancer types. Central and Eastern Europe experience the highest incidence rates (Ferlay et al., 2002). Smoking is the major cause of developing lung cancer (IARC, 2005). The combustion

of cigarettes can lead to the production of reactive oxygen species (ROS). Free radicals, components of ROS are found in cigarette mainstream and side stream smoke. Side stream sigarette smoke contains more toxic gases and free radicals than the mainstream cigarette smoke (Church *and* Pryor, 1985). The adverse effects of smoking may result from the accumulation of oxidative damage bought about by ROS, which is called oxidative stress (Avogbe, et al., 2005)

Oxidative stress is a condition that occurs due to imbalance between free radical and antioxidant productions. This will cause serious damage to biological macromolecules and disregulation of normal metabolism and physiological functions (Thomas, 2006)). Free radicals can cause lipid peroxidation in cell membranes, which in turn produces compounds that are toxic to cells, such as malondialdehyde (MDA). Elevated levels of MDA show the increased activity of lipid peroxidation (Mayne, 2003).

As a response to oxidative damage, antioxidants are produced. Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Antioxidants can be derived from the body or from outside the body. Antioxidants from outside the body are natural food ingredients from fruits. One of the antioxidant is mangosteen pericarp extract. Crude ethanol extract of mangosteen pericarp (CEMP) contains high concentration of antioxidants. The main natural antioxidants in pomegranate are polyphenols; and one of the polyphenols is the flavonoid.

5.4.1 MDA profile

There are several methods of examination to measure MDA, such as spectrophotometer and HPLC. Research conducted by Lovri showed no difference in the results by using the two methods. (Lovri et al., 2008)

A very low level of MDA was likely due to the age of the rats used that were very young i. e. about three weeks, while some studies used mice at the aged of 6-9 weeks on average. Elevated levels of MDA that showed the lipid peroxidation process in the three

groups were due to the exposure of various chemical substances containing cigarette smoke that included free radicals (Ueta et al., 2003).

In this study, rats were exposure to passive cigarette smoke. Cigarette smoke directly inhaled by active smokers is called mainstream smoke. Side stream smoke is the smoke generated from burning cigarettes. The side stream smoke contains more toxic gases and free radicals compared to the main smoke. This cigarette smoke can cause lipid peroxidation. (Church and Pryor, 1985).

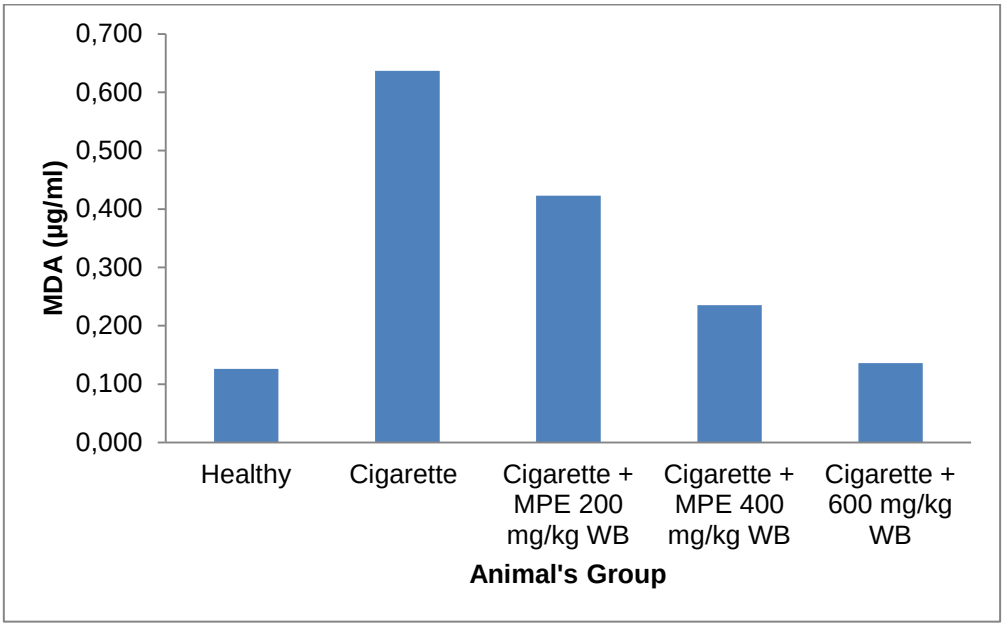

Figure 11. Malonaldehyde (MDA) levels after treatment, day-28. P = 0.000. ANOVA'S test.

Table 4. MDA levels after treatment. Post Hoc Tukey (α = 0.05)

Treatment	MDA (µg/ml)
Normal (P0)	0.126 a
Cigarette (P1)	0.637 b
Cigarette + 200 mg/kg MPE (P2)	0.423 c
Cigarette + 400 mg/kg MPE (P3)	0.235 d
Cigarette + 600 mg/kg MPE (P4)	0.136 a

*difference word showed the differences in one column

The mean MDA levels after treatment in all five groups were significantly different (P=0.000) as shown in Table 4. Increasing levels of MDA that showed the lipid peroxidation process in the four groups were due to the exposure of various chemical substances containing cigarette smoke that included free radicals (Ueta et al. 2003). In this study, rats were exposed to passive cigarette smoke. Cigarette smoke directly inhaled by active smokers is called mainstream smoke is the smoke generated from burning cigarettes. The side stream smoke contains toxic gases and free radicals compared to the main smoke. This cigarette smoke can cause lipid peroxidation.

In order to explain lowering-MDA level by CEMP consider to its richness in antioxidant capacity to counteract free radicals. The antioxidant activities of extract of *G. mangostana* pericarp evaluated by DPPH method was 5.94 µg/ml (Palakawong et al. 2010). The higher concentration of CEMP, the lower was the lipid peroxidation level.

In this study, the CEMP consumption in 200 mg/kg weighing of rat (P2), 400 mg/kg weighing of rat (P3), and 600 mg/kg weighing of rat (P4) noticeable effect could be obtained compared with control group. The study by Kaplan *et al*. (2002) in which pomegranate juice containing 0.175 mg flavonoids/day given to atherosclerotic mice was able to decrease macrophage lipid peroxidation. In another study, consumption of pomegranate juice that contained 0.035 mg flavonoids could reduce oxidative stress in atherosclerotic rats (Aviram et al. 2000). This difference is probably due to the exposure to cigarette smoke that led to higher oxidative stress conditions; so the intake of flavonoids have to be higher to be able to suppress the occurrence of lipid peroxidation.

Flavonoids contribute to counteract the free radicals in several ways. Some flavonoids work by inhibiting an enzyme that is responsible for the production of superoxide anions such as xantin oxidase. Moreover, it can also act as a scavenger of free radicals by donating electrons to superoxide radicals or lipid radicals to be stable (Smith et al. 2005).

5.4.2 Histopathological Observation

The histological examination of the lung of negative control showed normal structure of the lung (Fig. 12 a). Lung section of the positive control is remarkable changes, differences versus negative control (Fig. 12 b). When CEMP was given administrated in rats, some morphological changes was reduced and cells affected become few (Fig. c, d, and e).

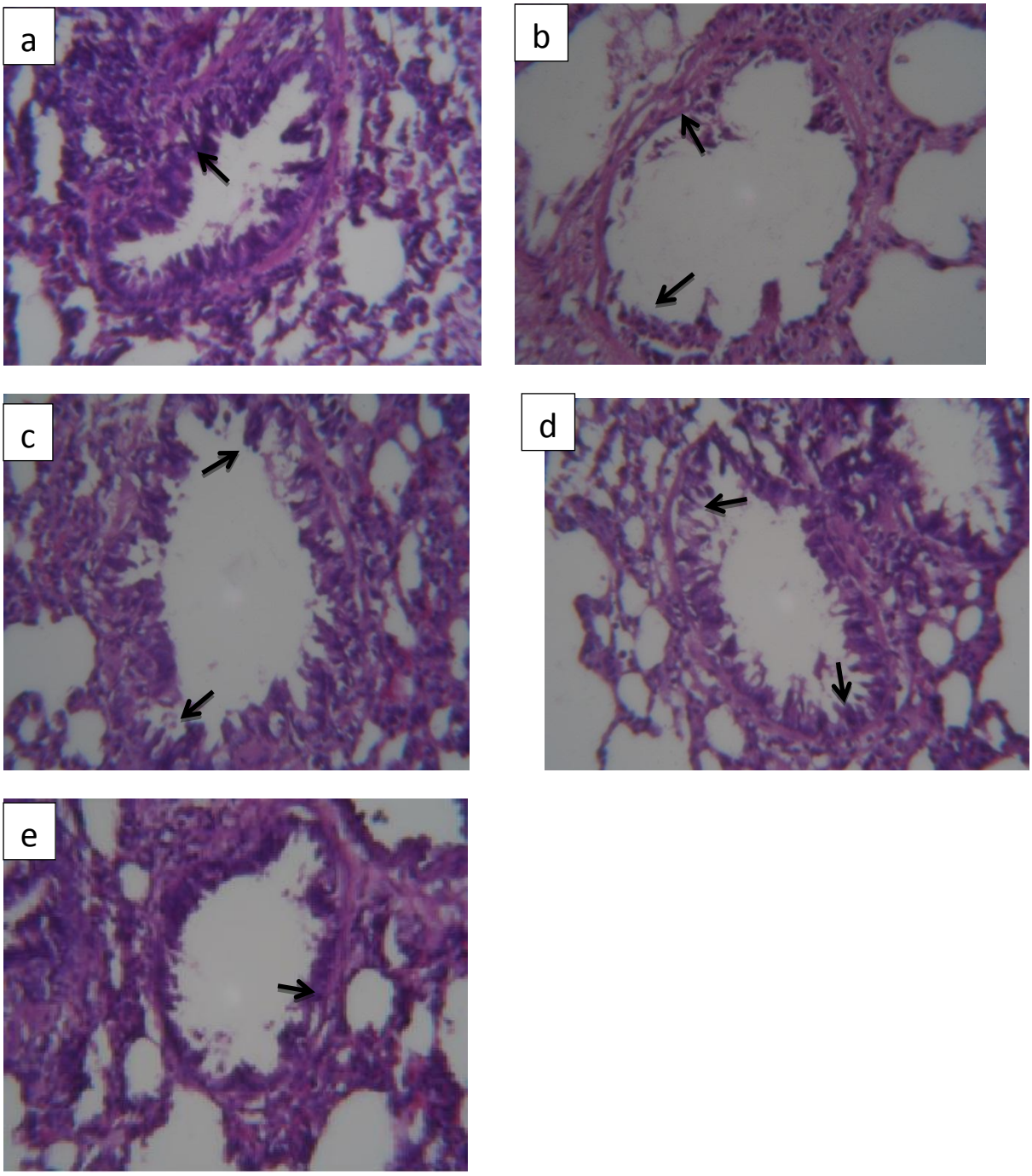

Fig. 12: Photomicrograph of the lung: (a) control rat; (b) exposure cigarette rat; (c) 200 mg/kg CEMP treated rat ; (d) 400 mg/kg CEMP treated rat; (e) 600 mg/kg CEMP treated rat.

The results of histophatological analysis demonstrated that cigarette smoking caused airspace enlargement. In our study, airspace enlargement increasing was observed after cigarette exposure without supplemented CEMP (Fig. 12 b) compared with normal control (Fig. 12 a), in agreement with Lee *et al* (2005) who also found that exposure of Sprague-Dawley rats to cigarette smoke caused a 74% increase in airspace enlargement after longer exposure. In contrast, Stevenson et al. (2007) did not show airspace enlargement until 8 months after cigarette exposure. The difference is likely due to the age of rat for the time of cigarette exposure.

The airspace enlargements were smaller than CE control in the groups that supplemented with CEMP (Fig. 12 c, d and e). The higher doses of CEMP, are the smaller airspace enlargements of the rat lung. It indicated that CEMP can protect the lung morphology after 3 weeks treatment. The precise mechanisms of the protective role of CEMP against CE-induced lung injury are currently unclear. CEMP contains of high antioxidant activity that to considered can improve the rat lung. In Chinese green tea model, antioxidant arrest multiple harmful mechanism of lung injury through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals generated from cigarette exposed (Klauning et al. 1999).

This research showed that the content of natural antioxidants in CEMP can counteract oxidative stress due to exposure to cigarette smoke. The limitation in our study are necessary to determine the type of antioxidants that most responsible for the results and difficult to discern in an in vivo study the cause and effect relationship between the beneficial effect of CEMP on lung structural damage.

Environmental tobacco smoke-induced early lung damage has also been found in healthy male adolescents (Rizzi et al., 2004). Our finding on lung damage from cigarette smoke exposure in younger rats corroborates with observations in humans. Smoking effects may diminish progressively with age in rats. Although the factors that affect lung maturity

are not well understood, lung morphogenesis is a highly regulated process that could be impaired by both genetic and environmental factors.

Our findings are important as our young rat model might provide a means of studying the mechanisms that control normal lung development and strategies for prevention of chronic obstructive pulmonary disease (COPD) in adult life. The other difference is likely to be the protocol for cigarette smoke exposure, which determines the amount of cigarettes taken, i.e. 12 cigarettes per day in this study. We also observed globlet cell hyperplasia in the epithelium of cartilaginous bronchi in all groups of rat, which might account for mucus hypersecretion in COPD patients (Lee et al., 2006).

Chinese green tea (Lung Chen) that high antioxidant compound was known probably arrests multiple harmful mechanisms of lung injury through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals generated from cigarette smoke exposure, in agreement with previous reports (Serafini et al. 1996; Klaunig et al., 1999). Consequently, we observed a protection of lung morphology after cigarette exposure by CEMP that also rich of antioxidant mainly flavonoids.

In conclusion, giving CEMP at the levels of 200, 400, and 600 mg/ kg weighing of rats could supress the occurrence of lipid peroxidation as indicated by MDA levels compared to control group.

VI. CONCLUSION

6.1 Conclusion

Based on the result of this study, it can be concluded that:

1. Phytochemical properties of extract ethanol of mangosteen pericarp (CEMP) contains of flavonoids, terpenoids, and alkaloids.
2. Treatments CEMP with doses 200; 400 and 600 mg/bw decrease the MDA levels of rats that exposure by cigarette.
3. The CEMP can repair the lung histology profiles of the rats that exposure by cigarette.

6.2 Suggestion

This research showed that the content of natural antioxidants in CEMP can counteract oxidative stress due to exposure to cigarette smoke. However, a more in depth examination of all antioxidant contents that are present in CEMP and their capacity needs to be conducted in future studies. These studies are necessary to determine the type of antioxidants that have significant roles in CEMP. This research did not shoe which antioxidant in CEMP were the most responsible for this results, and this fact was the limitation of this study.

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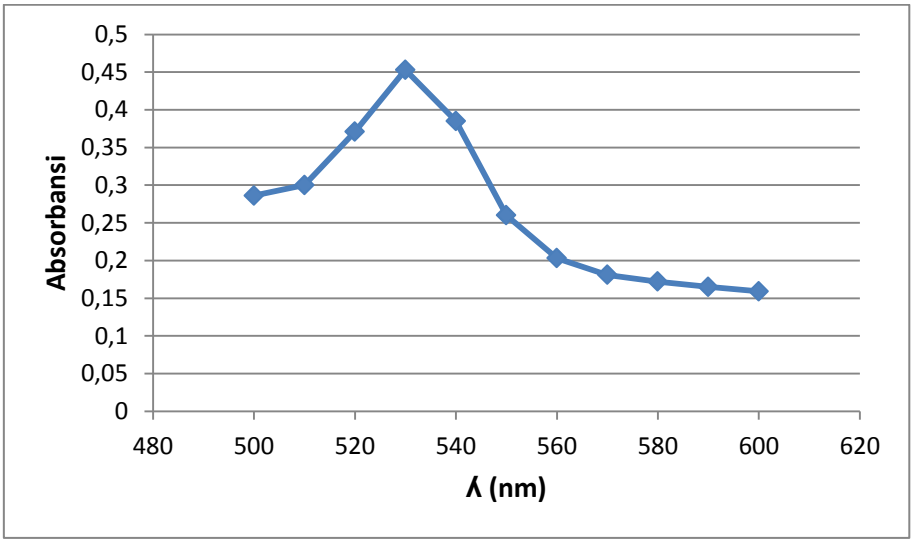
APPENDIX

1. Determining ʎ optimal

Tabel a. Standard MDA 4 ppm

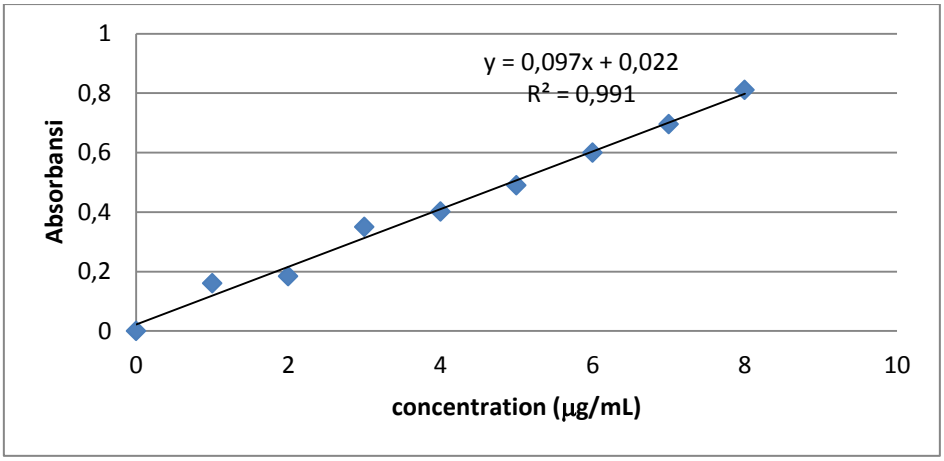
λ (nm)	Absorbansi
500	0.286
510	0.3
520	0.371
530	0.453
540	0.385
550	0.26
560	0.203
570	0.181
580	0.172
590	0.165
600	0.159



2. Curve MDA

Tabel b. Standard MDA λ maximum = 532 nm in different concentration

Standard concentration (µg/mL)	Absorbansi
0	0
1	0.16
2	0.184
3	0.35
4	0.401
5	0.49
6	0.6
7	0.695
8	0.811



y =0,097x + 0,022

Tabel c. Concentration MDA in Serum

Rats	Absorbansi	MDA levels (µg/mL)
Healthy	0.035	0.134
	0.031	0.093
	0.037	0.155
	0.033	0.113
	0.035	0.134
Average		0.126
Exposure with cigarette	0.085	0.649
	0.079	0.588
	0.087	0.670
	0.080	0.598
	0.088	0.680
Average		0.637
200mg/kgBB	0.062	0.412
	0.066	0.454
	0.060	0.392
	0.061	0.402
	0.066	0.454
Average		0.423
400mg/kgBB	0.039	0.206
	0.042	0.206
	0.045	0.237
	0.038	0.268
	0.037	0.258
Average		0.236
600mg/kgBB	0.023	0.134
	0.017	0.155
	0.020	0.175
	0.022	0.103
	0.019	0.113
Average		0.136

Tests of Between-Subjects Effects

Dependent Variable:MDA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.947[a]	4	.237	243.121	.000
Intercept	2.423	1	2.423	2.487E3	.000
MPE	.947	4	.237	243.121	.000
time * MPE	.000	0	.	.	.
Error	.019	20	.001		
Total	3.390	25			
Corrected Total	.967	24			

a. R Squared = .980 (Adjusted R Squared = .976)

Post-Hoc Tukey

MDA

Tukey HSD

perlakuan	N	Subset 1	2	3	4
normal control	5	.1258			
cigarette + 600 mg/kg MPE	5	.1360			
cigarette + 400 mg/kg MPE	5		.2350		
cigarette + 200 mg/kg MPE	5			.4228	
cigarette control	5				.6370
Sig.		.985	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .001.